ABSTRACT

Background: A semen analysis exam is a routine check that is done to evaluate fertility. The World Health Organization (WHO) recommended a manual method to obtain objective and standardized values. However, sometimes some errors can be found with this method such as motility. Computer-Assisted Sperm Analysis offers a way to reduce inaccuracies that often occur with manual methods.

Reviews: CASA systems consist of a microscope which connected into a camera to detect microscopic sperm suspension images and a computer installed with special software to extract desired information and produce the desired output. In the morphological examination, CASA can reduce the coefficient of variation (CV) which is around 4.8% compared to the manual examination but the time required is longer than manual. CASA can visualize and evaluate sperm kinematics. Various parameters such as mean path velocity (VAP), curved velocity (VCL), straight-line velocity (VSL), lateral head displacement amplitude (ALH), or beat cross frequency can be obtained, and this allows a detailed view into the behavior of individual sperm. The limitations affecting CASA's ability to provide accurate results for sperm concentration and percentages of motile or progressively motile spermatozoa.

Summary: CASA has several advantages through its ability to calculate more detailed parameters, but a qualified operator must operate it because there is some potential for misinterpretation. The combination of The Manual and CASA is highly recommended for better results.

Keywords: CASA, Semen Analysis, Sperm Analysis, Morphology, Motility

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INTRODUCTION

Semen analysis is a routine procedure that should be performed in male fertility evaluation. Word Health Organization recommends guideline in obtaining the most standardized and objective values using a manual method. Manual SA examination is a procedure that is directly carried out by humans following the 2010 WHO protocol, there is no machine involvement in ejaculate analysis. In contrast to automatic or using machines such as CASA or some other machine products, analysis using machines is entirely controlled by machines. Humans only prepare a sample and then put it into the machine then it will be read by the machine according to the program it has. Of Course in manual procedure, there are some conditions that often lead to false results in manual method. Doughlas et al stated several conditions that might result in false or error in manual procedure such as, interindivudual (observer) variation especially motility and morphology examinations which are closely related to subjectivity, then examiners who may not be trained and standardized, sampling errors, and andrology laboratories that is not standardized. The sperm motility assessment presents a challenge, not only in terms of the subjective analysis of gamete velocity, but also standardized temperature of the analysis or time elapsed between sample collection and the result.

Taking into account the inaccuracies in the manual examination processes, a computerized semen analysis with a good sample preparation method using appropriate hardware and software settings could improve the accuracy of semen analysis result. Here we describe some information that should be known about computerized semen analysis.

REVIEW

Definition

The acronym CASA represents, interchangeably in the literature, the terms “computer-aided sperm analysis” and “computer-assisted sperm analysis”. CASA systems consist of a microscope which connected into a camera to detect microscopic sperm suspension image and computer installed with special software to extract desired information and produce the desired output. Systems provide many values for motion or morphology of each spermatozoon studied.

Sperm morphology in manual and CASA method

Sperm morphology is positively associated with and able to predict fertilization in natural and assisted reproduction. Using 4% normal morphology as the cut off value, Premal et al concluded that sperm morphology impacted neither the pregnancy rate nor the live birth rate in couples undergoing intrauterine insemination. However, Erdem et al found that there was a difference in normal sperm morphology between cycles that did and did not result with clinical pregnancy and live birth. The best cut-off value for normal sperm morphology (%) to predict live birth was 4.5% in male sub fertile group. Another study by Sun et al revealed that the pregnancy rates per cycle were 7.60%, 12.67%, 13.62% and 13.13% in patients with <5%, 5–9%, 10–14% and >14% normal forms, respectively. Moreover, no pregnancies occurred in women >35 years old with normal sperm forms below 5%.

Li et al analyzed 4756 cases of infertility patients treated with conventional-IVF(c-IVF) or ICSI, which were divided based
on sperm normal morphology > 14%, 4%–14%, and 4%. The rate of fertilization, normal fertilization, high-quality embryo, multi-pregnancy and birth weight of twins gradually decreased significantly with the decrease in normal sperm morphology in the c-IVF group (P<0.05), while the miscarriage rate was significantly increased (p<0.01). We conclude that WHO 5th edition reference 4% is more applicable for IVF setting. Therefore, we apply PERSANDI reference value 5% for normal morphology for routine semen analysis in our laboratory.

Morphology provides the most independent and stable semen assessment parameters. However, manual sperm morphology assessment can be influenced by many factors, including technician experience. Wang et al studied the assessment variability by evaluators in the recognition of normal sperm and various sperm defects using the strict criteria recommended by the World Health Organization. They found that the coefficient variation (CV) of normal sperm were 4.80%. CASA might reduce this CV since it is performed with more objective measurement criteria. However, it should be noted that automatic morphology assessment is more time consuming than the manual analysis. But, the technician could record all image and evaluate them with another technician or supervisor in order to make a more valid result.

The WHO manual only classifies spermatozoa as normal or abnormal. However, it should be noted that an abnormal spermatozoa may have only one specific abnormality or any combination of two to four abnormalities. There are three indices for describing these multiple sperm anomalies. The multiple abnormalities index (MAI), used in the French modified David classification, is the average number of abnormalities per abnormal spermatozoa. The teratozoospermia index also reflects the mean number of abnormalities per abnormal spermatozoa, but a maximum of four abnormalities per abnormal spermatozoon are counted. The sperm deformity index (SDI) is the number of abnormalities divided by the total number of spermatozoa (normal and abnormal). Multiple sperm defect could be counted manually, but it is time consuming. CASA make it easier and faster to count MAI, TZI, and SDI.

The defining cut-off value for TZI is 1.64 for in vivo. A high TZI value of ≥ 1.90 can be regarded as a poor prognosis for normal IVF, and these patients should be taken directly to ICSI.

Sperm motility in manual and CASA method

CASA also able to visualize and evaluate consecutive images of sperms to obtain precise and valid information on the kinematics of individual sperms. Various parameters like average path velocity (VAP), curvilinear velocity (VCL), straight line velocity (VSL), amplitude of lateral head displacement (ALH), or beat cross frequency can be obtained, and this allows a detailed behavior of the individual sperms.
Youn et al found that the average path velocity (VAP) and percentage rapid movement before semen preparation were significantly different between the pregnancy and non-pregnancy groups of couple who performed intra uterine insemination ($p=0.033$ and $p=0.022$). Using a receiver operating characteristic curve to measure sensitivity and specificity, the optimal threshold value for the predictors of pregnancy was rapid movement $\geq 30.1\%$ before preparation for IUI.\textsuperscript{11}

The curvilinear velocity (VCL) is the average speed of a sperm head through its real path. The VCL is also important parameters in preliminary semen analysis for male infertility.\textsuperscript{12,13} Larsen et al found that VCL $> 25$ $\mu$m/s is the most significant and independent CASA parameter in predicting male fertility potential. Increasing number of sperm with VCL $> 25$ $\mu$m/s would increase cumulative probability of conception.\textsuperscript{13}

VSL is determined by finding the straight-line distance between the first and last points of the trajectory and correcting for time. This value then gives the net space gain within the observation period. Garret et al categorized VSL into three group; Low ($<30$ $\mu$m/s), Medium ($30 – <50$ $\mu$m/s), and High ($>50$ $\mu$m/s). They found that VSL are strongly related to pregnancy rates in sub fertile couples.\textsuperscript{4}

A new parameter for predicting the pregnancy have been proposed recently, the ratio of Mean Sperm Energy Index to Total sperm Energy Index (MEI/SEI). Isobe found that subjects with MEI/SEI $> 2$ were infertile.\textsuperscript{15} This new parameter needs ALH parameter which could be quantified by CASA.
Figure 3. Sperm energy index in the control and sterile groups. A scatter diagram with MEI plotted on the vertical axis and SEI on the horizontal axis in the sterile and control groups. All subjects with \((\text{MEI})/(\text{SEI}) > 2\) were in the sterile group.\(^{15}\)

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<tr>
<td>1</td>
<td>Multiple abnormalities index (MAI)</td>
<td>The average number of abnormalities per abnormal spermatozoon.</td>
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<td>2</td>
<td>The teratozoospermia index</td>
<td>The mean number of abnormalities per abnormal spermatozoa, but a maximum of four abnormalities (categories) per abnormal spermatozoon</td>
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<tr>
<td>3</td>
<td>The sperm deformity index (SDI)</td>
<td>The number of abnormalities divided by the total</td>
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<td>4</td>
<td>The average path velocity (VAP)</td>
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<tr>
<td>5</td>
<td>The curvilinear velocity (VCL)</td>
<td>The average speed of a sperm head through its real path.</td>
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<tr>
<td>6</td>
<td>The straight line velocity (VSL)</td>
<td>The average speed of sperm in straight-line distance between the first and last points of the trajectory and correcting for time.</td>
<td>&gt;50µm/s</td>
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**Sperm concentration in manual and CASA**

The sperm concentration is a critical component of semen analysis. Traditionally, the Improved Neubauer hemocytometer has been used for counting sperm concentration manually and consider as gold standard for studying other chamber.\(^{16-18}\) However, CASA system require another type of chamber for sperm measurement such as Makler Chamber and Leja.

The type of chamber used for CASA may have a considerable influence on the measurements of sperm concentration. When assessing the concentration in Makler chamber, it should be considered that the number of spermatozoa in a sample may be overestimated.\(^{19}\) Leja is a disposable capillary-loaded slides which commonly recommended to be used with most CASA systems. There is an error concentration measurement possibility that caused by the Segre-Silberberg (SS) effect, which occurs during Poiseuille flow in thin, capillary-loaded slides. The SS effect does not appear to have time to develop in the hemocytometer, which at 100 micron is
considerably deeper than most disposable slides.\textsuperscript{18}

It may be concluded that in order to obtain a valid sperm concentration value, CASA should be fulfilled with an appropriate chamber with adjustment of SS effect.

**CASA limitation**

The limitations affecting CASA’s ability to provide accurate results for sperm concentration and percentages of motile or progressively motile spermatozoa, fall into two major categories: biological and technical limitations.

Human semen is typically very “dirty”, containing lots of particles, and cellular and other debris (large amount of background noise). It also has a generally high viscosity which lead to difficulty in making accurate representative sampling. The operator should homogenize the semen well prior to CASA examination.

There are several problems in discriminating between spermatozoa and non-sperm objects, and between immotile and motile objects. CASA instruments cannot analyze flagellar beating directly and must rely on tracking the movement of the sperm head. Immotile objects with similar size and appearance to sperm heads could be detected as motile sperm. Moreover non-progressive sperm motility couldn’t be detected when movement of the flagellum is not able to be analyzed.\textsuperscript{20}

There are several improvements in order to make more reliable result. The positive phase contrast optics will reduce the misclassification of debris as sperm heads. Newer version of CASA result is able to be ‘cross checked’ by the operator so that the operator might add several sperm which are not detected automatically.

**CONCLUSION**

It may be concluded that CASA has several advantages through its ability to count more detail parameters, but there are some potencies of miss-interpretation. Using CASA doesn’t mean that operator only needs to put the sample and receive the result. He/she should keep the temperature of the semen, homogenize the sample well prior to examination, and cross check the result before it’s printed. Several seminal plasma examinations also could not be performed automatically until now. It means qualified operator is still required for semen analysis procedure.

**REFERENCES**


